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PATENT
Attorney Docket No: 28957/35061A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al. U.S. Serial No.: 09/427,657 Filed: October 26, 1999 For: Use of VEGF-C or VEGF-D Gene or Protein to Prevent Restenosis. Group Art Unit: 1636 Examiner: S. Kaushal	I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on _____, 2003. _____ David A. Gass

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. SEPPO YLÄ-HERTTUALA

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. I am a co-inventor of the above-identified U.S. Patent Application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the currently pending claims of the above-identified application.

2. The application as originally filed describes the use of VEGF-D polynucleotides or polypeptides to treat restenosis in a manner analogous to its description of the use of VEGF-C to treat restenosis. (See, e.g., Example 9 at p. 36.) Jula Rutanen (a co-worker) and I recently performed an experiment to demonstrate the efficacy of VEGF-D to inhibit restenosis by adenovirus-mediated gene transfer.

3. We performed balloon-denudation of the aorta on three sets of rabbits substantially as described in Example 1 on page 27 of the patent application. Balloon-

denudation results in intimal thickening and smooth muscle cell proliferation in the vessel, leading to stenosis of the vessel quantifiable by an increase in the intimal/media (I/M) ratio. This serves as an excellent animal model for restenosis following angioplasty.

4. As described in the patent application, the human prepro-VEGF-D polypeptide undergoes proteolytic processing to remove N-terminal and C-terminal propeptides, resulting in a mature VEGF-D comprised of residues 93-207 of SEQ ID NO: 4. (See patent application at p.20, lines 17-25.) For our experiment we prepared (codons 93-201)an adenovirus vector containing a cDNA encoding a mature human VEGF-D polypeptide operably linked to a cytomegalovirus promoter and a SV40 polyadenylation signal sequence.

5. The gene transfer was preformed essentially as described in Example 1 on page 27 of the above-identified application.

6. The rabbits of the first study group were sacrificed six days after the gene transfer occurred and examined. The intima/media ratios (I/M) were similar and low for both VEGF-D and *LacZ* treated control animals in this group (I/M 0.16 ± 0.01 for VEGF-D and 0.15 ± 0.01 for *LacZ*), indicating that no significant neointimal thickening had yet occurred.

7. The second study group of rabbits were sacrificed two weeks after the gene transfer occurred and examined. The rabbits from this group that received the VEGF-D adenoviral gene therapy showed a significantly decreased I/M compared to the *lacZ* control rabbits (I/M 0.21 ± 0.05 for VEGF-D and 0.44 ± 0.07 for *lacZ*, $P < 0.05$).

8. The third study group of rabbits were sacrificed four weeks after the gene transfer occurred and examined. The I/M ratio for the VEGF-D treated animals remained lower than in the control animals, although the treatment effect was no longer statistically significant at four-week time point (IM 0.47 ± 0.13 for VEGF-D and 0.63 ± 0.10 for *lacZ*, *P*

= NS). We attribute this loss of a statistically significant difference to the transient nature of the singly-administered adenoviral gene transfer agent.

9. The foregoing data demonstrates that single administration of adenovirally-mediated VEGF-D gene transfer significantly reduced intimal thickening after two weeks of treatment, an effect that appeared to persist at the four-week time point. The data indicates therapeutic utility for VEGF-D in the prevention of post-angioplasty restenosis.

Certification

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

12/5/03
Date

Seppo Ylä-Herttuala